Keywords: Curcumin, Oxidative stress, Type 1 diabetes, Liver, Polyol pathway, Protein kinase C

1 Introduction

Diabetes mellitus (DM) is one of the most common endocrine metabolic disorders, which leads to glucose toxicity by increasing protein glycation and activating the polyol pathway associated with the protein of poly ADP ribose polymerase (PARP) and protein kinase C expression [1-5]. Normally, DM can generate a lot of reactive oxygen species (ROS) which can cause oxidative stress [6-9], resulting in inflammation, cell death and system organ dysfunction [10,11]. Previous studies have shown that the redox balance is disrupted by activating the polyol pathway and PARP [5,12]. Firstly, the glucose was transformed to fructose using NADH as coenzyme in the polyol pathway, leading to NADPH converting to NADH and over-production of the consumption of glucose [13,14]. Secondly, NADH was dehydrogenated to produce NAD+, which was used as a substrate to over-activated PARP resulting in the DNA oxidative damage in diabetes [15]. Therefore, restored redox balance would decrease levels of NADH and maybe alleviate the oxidative stress [16]. In addition, cells defend themselves by superoxide dismutase (SOD), glutathione peroxidase (GPx) and reduced glutathione (GSH) to combat ROS damage [17,18].

Curcumin is extracted from the rhizome of turmeric and has been shown to have many bioactivities involved in antioxidant, anti-inflammatory and metabolic disorders in different biological systems and animal models [22-27]. Given its beneficial effects, safety and cost-effectiveness, curcumin could be used in diabetes and its resulting complications as well as for obesity-related metabolic
Curcumin attenuates oxidative stress in liver in Type 1 diabetic rats

2 Materials and methods

2.1 Chemicals

Streptozotocin (STZ) and curcumin were obtained from Sigma Chemicals Company (USA). The blood glucose, malondialdehyde (MDA), superoxide dismutase (SOD), sorbitol dehydrogenase (SDH), glutathione peroxidase (GPx) and reduced glutathione (GSH) kits were purchased from Nanjing Jiancheng Biologic, Inc. (Nanjing, China). Glucagon radioimmunoassay kit was procured from North Institute of Biological Technology (Beijing, China). The aldose reductase (AR), glucose-6-phosphate dehydrogenase (G6PD), glycogen synthetase (GS) and glucose-polymerizing enzyme (GPE) kits were purchased from BioAssay (Hayward, CA). Amplite colorimetric total NADP and NADPH assay kit was purchased from AAT Bioquest, Inc. (Sunnyvale, CA). All the other chemicals were of analytical grade.

2.2 Animals and ethics statement

Forty-five seven-week-old male Sprague-Dawley rats were obtained from Model Animal Research Center of Nanjing University (Nanjing, China). All rats were acclimatized to laboratory conditions, which included temperature (22±2 °C) and a 12 h light/dark cycle and were maintained on standard food pellets and tap water for one-week.

Ethical approval: All animal experimental procedures were approved by Fujian Agriculture and Forestry University Animal Care and Use Committee and the law of laboratory animals of the Fujian province Zoological Society.

2.3 Experimental design

All rats were weighted and randomly assigned; group I (NC, n=15) was a control group, group II (DC, n=15) was a diabetes control group, and group III (Cur, n=15) was a treated group. STZ in fresh 0.1 mol/L citrate buffer (pH 4.5) was administered intraperitoneally at a single dose of 80 mg/kg to establish diabetic rat model [31]. The control rats were also intraperitoneally administered with the citrate buffer. After two days, fasting blood glucose was tested from the tail vein of rats. Rats with diabetes were confirmed after fasting blood glucose levels exceeded 11.1 mmol/L. The curcumin treated group was put on a normal diet plus 1.5 g curcumin/kg body weight administered orally by intragastric intubation daily for 21 days [31-33].

2.4 Sampling

At the end of the experiment, animals were left to fast overnight and sacrificed by cervical decapitation after administering ether anesthesia. Blood was collected in heparin sodium vessels. Plasma was collected by centrifugation at 3500 rpm for 15 min and glucose concentration, MDA, SOD, SDH, GPx and GSH activities were measured. The liver was immediately collected and cleared of the adhering fat, weighed and homogenized, and then stored at -80°C until analysis.

2.5 Analytical methods

Blood glucose levels and plasma concentrations of MDA, SOD, SDH, GSH and GPx were determined using chemical kits according to the manufacturer’s instructions. The activities of SDH, GSH and GPx in the Liver were measured from homogenates. Liver tissue homogenates and hemolysates were prepared in Tris-HCl buffer (0.4 mol/L; pH 7.0). When testing the liver SDH, an aliquot of liver homogenate was treated with 1.6 mL triethanolamine buffer (0.2 mol/L; pH 7.4) and 0.2 mL NADH (12 mmol/L).

Glucagon in the liver was measured using a radioimmunoassay kit according to the manufacturer’s instructions. Samples, standards and controls were incubated with 125I-Glu and rabbit anti-Glu antibody for 24 h, and then they were separated by centrifugation. A separating medium was removed and the sediment was saved for measurement on a γ-counter. The activities of AR, G6PD, GPE and GS in the liver were measured spectrophotometrically using chemical kits according to
the manufacturer’s instruction. A decrease of NADPH’s absorption or monitoring NADPH production at 340 nm was used to analyze AR activity and G6PD activity, based on coupling its production of glucose-6-phosphate to NADPH production.

### 2.6 SDS-PAGE and western blot analysis

Protein was extracted from frozen liver tissue. Protein concentration was measured using bovine serum albumin as the standard in the Bradford assay and 50 µg protein samples were heat denatured. Subsequently, the SDS-PAGE (10% gel) was performed to separate proteins according to standard procedures [34]. The protein antibody of anti-poly ADP ribose polymerase antibody (PARP, 1:1500, Abcam) and anti-protein kinase C antibody (PKC, 1:1500, Abcam) was used. The actin (1:10000, Abcam) was used as an internal control. Finally, the blot was washed and detected by enhanced chemiluminescence (ECL) using the LumiGlo substrate (Pierce, USA) and Clarity Western ECL Substrate (BioRad, USA).

### 2.7 Statistical analysis

The results were evaluated by one-way analysis of variance using the SPSS-20.0 software followed by LSD. Data was expressed as mean ± SE and statistical significance was considered when P<0.05.

### 3 Results

#### 3.1 Blood glucose, plasma SOD and MDA concentration.

As shown in Table 1, after 21 days of experiment, the blood glucose level was significantly increased in the DC group (P<0.001) and Cur group (P<0.05) compared with the NC group. However, curcumin-treated diabetic rats had significantly reduced blood glucose levels (P<0.001). Compared to the NC group, the level of MDA was significantly increased in the DC group (P<0.001), whereas this change was attenuated in the curcumin-treated diabetic rats (P<0.01). Furthermore, an obvious reduction of SOD activity in the DC group compared with the NC group (P<0.01), but in the Cur the SOD activity was increased significantly compared with that in the NC group (P<0.05) and DC group (P<0.001).

#### 3.2 Hepatic glycogen content, activities of SDH, GPx and GSH in the liver and plasma

As shown in Table 2, the level of hepatic glycogen in the DC group was lower than that in the NC group (P<0.01), but curcumin treatment significantly increased the hepatic glycogen levels compared with the DC group (P<0.01). Data in Table 2 also includes the concentrations of SDH, GPx and GSH activities in the liver and plasma.

### Table 1. Blood glucose, plasma levels of MDA and SOD

<table>
<thead>
<tr>
<th>Item</th>
<th>Glucose (mmol/L)</th>
<th>MDA (nmol/mL)</th>
<th>SOD (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC1</td>
<td>6.01±2.47</td>
<td>6.03±1.03</td>
<td>25.01±3.47</td>
</tr>
<tr>
<td>DC2</td>
<td>17.99±1.31</td>
<td>9.22±0.67</td>
<td>18.76±2.45</td>
</tr>
<tr>
<td>Cur3</td>
<td>8.98±2.36</td>
<td>6.38±0.24</td>
<td>28.66±1.09</td>
</tr>
</tbody>
</table>

Note: the results are from an analysis of eighteen rats in each group. Data is presented as Means ± SE, indicates, * P<0.05, ** P<0.01 and *** P<0.001 means differences compared with NC group; ## P<0.01 and ### P<0.001 means differences compared with DC group.

1 NC means control group; 2 DC means diabetic control group; 3 Cur means treated group which plus 1.5 g curcumin/Kg body weight given orally by intragastric intubation daily.

### Table 2. Hepatic glycogen (HG) levels, activities of sorbitol dehydrogenase (SDH), glutathione peroxidase (GPx) and reduced glutathione (GSH) in the liver and plasma in type 1 diabetes rats

<table>
<thead>
<tr>
<th>Item</th>
<th>HG (mg/g)</th>
<th>Liver SDH (Unit/g)</th>
<th>Plasma SDH (Unit/mL)</th>
<th>Liver GPx (µg glutathione / min●mg)</th>
<th>Plasma GPx (µg glutathione / min●mg)</th>
<th>Liver GSH (µg/mg)</th>
<th>Plasma GSH (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC1</td>
<td>11.22±1.82</td>
<td>4.05±0.18</td>
<td>3.86±0.08</td>
<td>11.75±0.18</td>
<td>14.98±0.68</td>
<td>23.61±1.18</td>
<td>22.86±1.39</td>
</tr>
<tr>
<td>DC2</td>
<td>5.62±1.26</td>
<td>8.28±0.43</td>
<td>5.55±0.74</td>
<td>6.58±0.13</td>
<td>10.35±0.23</td>
<td>10.72±0.43</td>
<td>13.55±0.91</td>
</tr>
<tr>
<td>Cur3</td>
<td>7.24±1.07</td>
<td>5.23±0.17</td>
<td>4.01±0.08</td>
<td>11.23±1.23</td>
<td>14.35±0.71</td>
<td>21.07±0.81</td>
<td>20.77±0.21</td>
</tr>
</tbody>
</table>

Note: the results are from an analysis of eighteen rats in each group. Data is presented as Means ± SE, indicates, * P<0.05, ** P<0.01 and *** P<0.001 means differences compared with NC group; # P<0.05, ## P<0.01, ### P<0.001 means differences compared with DC group.

1 NC means control group; 2 DC means diabetic control group; 3 Cur means treated group which plus 1.5 g curcumin/Kg body weight given orally by intragastric intubation daily.
of all animals. The SDH activity in the DC group and Cur group was significantly higher (P<0.01) than that in the NC group. Curcumin treatment significantly decreased the activity of SDH in both the liver and plasma (P<0.01) compared with those in the DC group. The values of GPx and GSH were obviously decreased in both the liver (P<0.001, P<0.001, respectively) and plasma (P<0.01 and P<0.05, respectively) in the DC group compared with the NC group. Furthermore, curcumin treatment significantly increased the levels of GPx and GSH in the liver (P<0.001 and P<0.001, respectively) and plasma (P<0.01 and P<0.001, respectively) compared with those in the DC group.

3.3 Redox imbalance parameters associated with liver enzyme activity

As results in Figure 1A demonstrate, the activity of aldose reductase was noticeably elevated in the DC group, but this change was inhibited in the Cur group. The enzyme systems convert glucose to fructose and NADPH to NADH, resulting in the significant decrease of NADPH (P<0.001) in the DC group when compared to the NC group (Figure 1B). In addition, NADPH content was lower, so GSH content was decreased as well. The activity of G6PD in the DC group was lower than in the NC group (Figure 1C), and the results suggested that decreased levels of NADPH could be partly driven by decrease of the G6PD activity. As shown in Figure 1D and 1E, the activities of glucose-polymerizing enzyme and glucose synthetase were reduced significantly in the DC group compared with the NC group (P<0.01, P<0.001, respectively). The curcumin treated diabetic rats notably increased the activities of glucose-polymerizing enzyme and glucose synthetase (P<0.05, P<0.01, respectively), but they were still lower than in the NC group.

3.4 The protein expression of the PKC and PARP

As shown in Figure 2A and 2B, the protein contents of PKC and PARP were notably increased in the DC group compared with the NC group (P<0.01, P<0.001,
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the NC group, but the blood glucose level was attenuated significantly in the Cur group (Table 1). This result is consistent with the conclusions of previous research that showed dietary supplements for curcumin could regulate glucose levels [22,35]. In this study, the results showed that curcumin treatment significantly increased the hepatic glycogen level compared with the DC group (Table 2), which is ascribed to the activities of glucose-polymerizing enzyme and glucose synthetase (Figure 1D and 1E). Previous studies have reported stress-sensitive kinases to be involved in insulin receptor substrate-mediated insulin resistance in diabetes, including several isozymes of PKC-β and PKC-γ [36,37]. Activation of PKC isoforms are able to phosphorylate multiple targets associated with glycogen decomposition [2], along with increased hexosamine pathway flux [37]. In this study, curcumin treatment obviously down-regulated the PKC protein expression, suggesting that curcumin respectively). The protein expression of PKC and PARP was significantly down-regulated in the Cur group compared with the DC group (P<0.001, P<0.05, respectively) except of the protein content of PARP in the Cur group that was still obviously higher than that in the NC group (P<0.01).

4 Discussion

In this study, the major findings are described in the following section. Firstly, curcumin attenuated the NADH/NAD⁺ redox imbalance in diabetic rats by inhibiting the polyol pathway and PARP. Secondly, curcumin treatment noticeably inhibited the PKC protein expression and increased the activities of glucose-polymerizing enzyme and glucose synthetase, resulting in a decreased level of oxidative stress. In our experiments, the blood glucose was markedly elevated in the DC group compared with the NC group, but the blood glucose level was attenuated significantly in the Cur group (Table 1). This result is consistent with the conclusions of previous research that showed dietary supplements for curcumin could regulate glucose levels [22,35]. In this study, the results showed that curcumin treatment significantly increased the hepatic glycogen level compared with the DC group (Table 2), which is ascribed to the activities of glucose synthetase and increased glucose-polymerizing enzyme (Figure 1D and 1E). Previous studies have reported stress-sensitive kinases to be involved in insulin receptor substrate-mediated insulin resistance in diabetes, including several isozymes of PKC-β and PKC-γ [36,37]. Activation of PKC isoforms are able to phosphorylate multiple targets associated with glycogen decomposition [2], along with increased hexosamine pathway flux [37].

Figure 2. The of the PKC and PARP protein levels and a diagram of curcumin attenuate oxidative stress. A, protein kinase C (PKC); B, poly ADP ribose polymerase (PARP); C, a diagram of curcumin attenuate oxidative stress. Data is presented as Means ± SE, *, * P<0.05, ** P<0.01 and *** P<0.001 means differences compared with NC group; # P<0.05, ## P<0.01, ### P<0.001 means differences compared with DC group. The abbreviations are as following: OS, oxidative stress; MDA, malondialdehyde; SOD, superoxide dismutase; GSH, reduced glutathione; GPx, glutathione peroxidase; SDH, sorbitol dehydrogenase.
could increase the synthesis of glycogen regulator of blood glucose homeostasis.

A previous study demonstrated that oxidative stress could be prevented by the scavenging activity of antioxidant enzymes such as SOD and GSH [29,35,38-40]. GSH plays a critical role in regulating the intracellular redox system to defend cells from oxidative stress [41]. Recently, reports have shown that increased SOD and GSH activity could protect pancreatic β-cells from oxidative stress during diabetic conditions [6,42]. In the present study, plasma MDA was significantly increased in the DC group compared to those in the control group, indicating antioxidant defense system impairment. The activity of SOD was enhanced in the present study and may contribute to the protective effect of curcumin in diabetic rats by scavenging oxygen free radicals or enhancing the antioxidant capacity [6,42]. During diabetic condition, free radical production was increased due to decreased activity of SDH in the plasma and liver, revealing the susceptibility to free radical damage [46]. Administration of curcumin to diabetic rats could attenuate these changes. Our study demonstrates that not only NADPH levels were increased but also G6PD activity and GSH levels were elevated in the curcumin-treated rat model. It seems that the increase in NADPH content in the Cur group (Fig. 1B) would increase GSH formation because NADPH is required for GSH formation from GSSG by glutathione reductase [47]. Our results showed that G6PD activity, NADPH and GSH levels in the Cur group were higher than in the DC group. These results are similar to the previous descriptions [48-52].

SDH is an important enzyme in the polyol pathway, which catalyzes the conversion of sorbitol to fructose in the presence of NAD. Previous results have shown that the activity of SDH was elevated in the diabetic, leading to increased availability of fructose and better substrate than glucose for glycosylation [1]. In the present research, the activity of SDH in the plasma and liver was decreased in the Cur group compared with the DC group. These changes can decrease availability of sorbitol to inhibit the cascade amplification effect of oxidative stress via negative feedback regulation of the SDH activity in diabetic rats. These results imply that curcumin may prevent diabetic pathological conditions by enhancing glycogen synthesis and reducing hyperglycemia in the diabetic rat model.

In conclusion, a diagram illustrating the proposed mechanism of action of curcumin is shown in Figure 2C. We propose that the effect of curcumin attenuates oxidative stress in STZ-induced diabetes by inhibiting polyol pathway, PARP expression and down-regulating expression of protein kinase C.

Authors and Contributors: Substantial contributions to the conception or design of the work: ZL Xie; The acquisition data for the work: XQ Zeng, XQ Li, BB Wu, GZ Shen and QY Wu; Analysis data for the work: ZL Xie, XQ Zeng, XQ Li, BB Wu, GZ Shen and QY Wu; Interpretation of data for the work: ZL Xie; Drafting the work: ZL Xie and BB Wu; Revising it critically for important intellectual content: XQ Zeng, XQ Li, BB Wu, GZ Shen and QY Wu; Revised the manuscript: CB Wu. Final approval of the version to be published: ZL Xie, XQ Zeng, XQ Li, BB Wu, GZ Shen, QY Wu and CB Wu.

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Conflict of interest: Authors state no conflict of interest

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